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Procter&Gamble

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U.S. Environmental Protection Agency
Office of Pollution Prevention and Toxics
Document Control Office (7407), Rm. G-099
401 M St., SW
Washington, DC 20460

Subject: Comments on Proposed Test Rule for Hazardous Air Pollutants
Docket Number OPPTS-42187A; FRL-4869-1

Dear Sir or Madame:

The Procter and Gamble Company appreciates the opportunity to review and provide comments to the Environmental Protection Agency on the Proposed Test Rule for Hazardous Air Pollutants. We commend the EPA on the effort that has been put forth in updating the safety information on hazardous air pollutants.

The Proposed Test Rule has been reviewed by a Procter and Gamble's Corporate immunotoxicologist who has responsibility for assuring the safety of Procter and Gamble's ingredients and products.

We provide the following general comments for your consideration.

(1) We support the Agency's initiative to look for opportunities to update and to integrate new or improved toxicity test methods that have been appropriately validated (as outlined in the NIEHS/ICCVAM Validation and Regulatory Acceptance Guidance Document) into the new guidelines. However, if new methodology is proposed, it is critical that the methods have been appropriately validated and provide additional benefit e.g. more efficient, and/or more predictive of the toxicity endpoint.

For example, we have concern that some of the new methods that have been integrated into the immunotox guideline have not been validated sufficiently so as to meet the general validation criteria outlined by NIEHS/ICCVAM. In addition, it has not been demonstrated that the immune function assays being proposed in the revised 870-Health Effects guidelines provide any additional productive immunotoxicity data than that currently provided by immune tissue organ weight and histopathology. In view of this, we encourage the Agency to re-evaluate the appropriateness of integrating immunotoxicity functional assays into the immunotoxicity guidelines.

(2) We encourage the EPA to ensure that the revised 870 Health Effects immunotox guidelines are consistent with the on-going OECD harmonization of hazard classification and communication activities.

More specific comments on the immunotoxicology test guidelines are provided in the attached document. If the EPA has any questions regarding the comments provided, please call me at 513-627-1360.

Sincerely,
The Procter and Gamble Company
Elizabeth E. Sikorski
Corporate P&RS

cc: Sally D. Gasior
The Procter & Gamble Company
Corporate Regulatory & Government Affairs

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Elizabeth E. Sikorski
Procter & Gamble

Comments on 40 CFR Part 799: Proposed Test Rule for Hazardous Air Pollutants

These comments concern immunotoxicity testing proposed in 40 CFR Part 799. We are in agreement with the EPA's recommendation not to do comprehensive immunotoxicity testing. However, there are comments that were submitted to the EPA on the OPPTS guideline 870.7800 that we would like to reiterate regarding their use as a guideline for immunotoxicology testing in the proposed HAPs rule. It is important to consider whether the recommendations in 870.7800 are appropriate for this HAPs rule. Below are some of my concerns with these recommendations.

1. DEBATE ON HISTOPATHOLOGY AND HAZARD IDENTIFICATION

The development of the OPPTS 870.7800 guidelines are based on the premise "that certain endpoints done as part of routine toxicity testing are not sufficient to predict immunotoxicity". The reference cited to substantiate this statement is Luster et al. 1992 and 1993. The question of using routine endpoints to identify an immunotoxicant is still a hotly debated topic and is not resolved as indicated in the ILSI workshop summary (Neumann et al. 1995). After careful review of the Luster papers, it is not clear that their use as the sole scientific support for this position is adequate. It is important that this position by the EPA is supported by scientific data showing that standard toxicology parameters are not predictive of immunotoxicity. The studies summarized in Luster et al. 1992 were not all carried out at conditions recommended by OECD and ECETOC. **One**, some studies were done using doses below the MTD which is contrary to what the current proposed EPA guidelines recommend (page 4). If chemicals were re-evaluated with the highest dose at or near MTD, nonfunctional endpoint may be predictive. **Two**, some studies did not include histopathology of the bone marrow or draining and distal lymph nodes as recommended by the OECD and ECETOC. **Three**, route and time of exposure were not adequate for all compounds. Therefore, some of these studies evaluated in the Luster papers were not definitive studies for that chemical.

On page 202 of Luster et al. (1992), it states that "gross changes such as thymus weights or spleen cellularity appeared to be unreliable indicators of immunotoxicity". However, there are several points that we would like to make showing this statement is not adequately supported. **(1)** In the paper, it states that two assays have the highest association with immunotoxicity, namely, the PFC response and surface markers. It should be pointed out that the paper does not say these are the most predictive assay. These two assays have individual concordances of 78 and 83, respectively, and a pairwise concordance of 91 for 23 chemicals. We would like to point out that if the Agency consider thymus/BW ratio and PFC response they have individual concordances of 68 and 78 respectively, but the pairwise concordance is 92 (higher than for PFC and surface markers) and for 38 chemicals. Therefore, why add a more expensive, less validated assay (NK cell assay or flow cytometric analysis of the spleen) when it appears histopathology is as good or possibly better as the second assay? **(2)** In Luster et al. (1992), each endpoint is being statistically evaluated alone or paired with another endpoint. It may be that each endpoint alone or in combination with one other endpoint may be unreliable, however, decisions regarding potential toxicity are made using a compilation of various endpoints and not on one or two endpoints. It is our belief that an acute toxicity study that includes histopathology, hematology, and organ weights contain many endpoints that when assessed and evaluated together will identify an immunotoxic hazard. **(3)** In the Appendix of Luster et al. 1992, there are some immunotoxicants that produce changes in thymus or spleen weight or spleen cellularity without effects on the PFC assay. When used properly, standard toxicity endpoints are an integral part of the risk assessment and hazard identification processes.

In support of non-functional immune parameters in immunotoxicity hazard identification, an ECETOC committee has published two manuscripts, one discussing hazard identification (Basketter *et al.* 1994a) and the other discussing risk assessment (Basketter *et al.* 1994b). In Basketter *et al.* (1994a), it is recommended that Tier 1 testing "will provide a reliable and accurate means of identifying at an early stage potential immunotoxic effects". Tier 1 testing includes total and differential blood counts, spleen and thymus weight and histopathology and draining and distal lymph node and bone marrow histopathology. Immune function assays are included in Tier II and are not recommended unless indications of immunotoxicity are seen in Tier I. OECD guidelines (1992) are similar to recommendations by the ECETOC committee. Therefore, the OPPTS guidelines are in contrast to these published recommendations.

It is important to consider that companies evaluate new chemicals using extensive safety programs that consist of studies from 14 days to two years. It seems unnecessary to increase the number of animals, time and money for hazard identification if no additional benefit is gained.

2. RECOMMENDED IMMUNE FUNCTION ASSAYS HAVE NOT BEEN ADEQUATELY VALIDATED AND DON'T MEET CRITERIA EPA OUTLINES IN DOCUMENT

In the OPPTS 870.7800 guidelines, it states that immune function assays were chosen because they (1) accurately predict immunotoxicity, (2) are readily reproducible and (3) are easily incorporated into routine toxicity testing. The two primary immune function assays recommended in these guidelines, namely, the NK cell assay (particularly in the rat) and flow cytometric analysis at 90 days do not meet these criteria and have not been properly validated. The EPA should provide scientific justification for inclusion of the immune function assays, particularly the NK cell assay in the rat and flow cytometric analysis of the spleen.

A. NK Cell Assay

Assessment of NK cell number by flow cytometric analysis is not a common procedure, particularly in the rat. NK cell numbers in the spleen are generally very small which makes it difficult to conclusively assess changes. In addition, baseline values are highly variable from experiment to experiment making interpretation difficult at times. To our knowledge there are no published studies showing it is predictive of immunotoxicity or reproducible except Luster et al. 1992 and there are several critical problems with the interpretations of that manuscript. It is important for the EPA to cite references that show inclusion of this assay in the guidelines is warranted.

B. Flow Cytometric Analysis

There are no review articles or interlaboratory validation studies that demonstrate flow cytometric analysis of rat spleen can accurately predict immunotoxicity particularly at 90 days in the rat. Currently several laboratories (organized by Dupont) are involved in an inter-laboratory evaluation of spleen markers in the rat but this project is just beginning. Have validation studies been done to show that immunotoxicants that target T or B cells alter numbers or percentages of T or B cells, respectively? How many nonimmunotoxicants have been evaluated using flow cytometry? There is no doubt that flow cytometric analysis is very helpful in pinpointing or examining the mechanisms of an immunotoxic response but there is not enough information available to warrant inclusion in guidelines for hazard identification.

The proposed OPPTS guidelines also suggested use of spleen cells or peripheral blood lymphocytes (PBL) in flow cytometric analysis. However, there is no data correlating changes in PBL phenotypes with immunotoxicity.

The idea that analysis of surface markers using flow cytometry may be useful as a predictive assay for immunotoxicity appears to be due conclusions made in Luster et al. (1992). There are two problems with the studies evaluating surface markers as immune function assays in that article: (1) Twenty four assays using Thy 1.2 and sIg^+ were evaluated with a sensitivity of 47 and 46% and individual concordances of 67 and 64%, respectively (Table 3; Luster et al. 1994a). Only nine assays were done assessing $CD4^+$, $CD8^+$ and $CD4^+/CD8^+$ giving a sensitivity of 50, 75 and 100% and concordances of 78, 89, 100. The highest concordances were seen with CD4, CD8 and the CD4/CD8 ratio but there is very little data included in the statistical analysis. Therefore, considering each of these endpoints separately there is either (1) not enough data to show the predictability or reproducibility of those endpoints (CD4, CD8 and CD4/CD8 ratio) or (2) concordances equal to other nonfunctional immune parameters (Thy 1.2 and sIg). (2) In Luster et al. 1992, the different surface markers were grouped as one endpoint giving added weight to this endpoint. The chance of hitting one significant value using a number of endpoints is high. Therefore, statistical analysis of surface markers by grouping them together was not appropriate.

C. The guidelines recommend immune function assays that have only been evaluated by one to two laboratories but not adequately validated.
It seems as if the recommendations in the OPPTS 870.7800 come from individuals and the personal experiences of a few laboratories. Even though these laboratories do excellent work, there needs to be peer reviewed published compilations of the data that support the EPA recommendations. The data that is currently available is sound but there just doesn't seem to be enough on which to base a guideline which recommends the inclusion of new assays. For the immunotoxicology community, there are no reviews available showing interlaboratory validation studies available on (1) the NK cell assay or (2) flow cytometric analysis in rats and mice. Yet these assays are being included in guidelines. It is important to cite references that show these assays are reproducible or predictive.

Please see attached document from ICCVAM discussing validation and regulatory acceptance of toxicology assays. The Agency will see that neither assay, the NK cell assay or flow cytometric analysis of the spleen, meet the criteria outlined by ICCVAM. The validation requirements seen in areas like eye irritation are not being applied to immunotoxicology testing. The literature needs to be reviewed and summarized so decisions regarding immune function assays can be based on peer-reviewed scientific evidence.

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EXECUTIVE SUMMARY

Toxicological test methods are being developed and revised with increasing frequency. Scientists seek methods that will provide improved assessment of the potential toxic effects of chemicals and other agents to human health and the environment, evaluate new important toxicity endpoints, incorporate current scientific understanding of toxic mechanisms, can be conducted in less time and with less expense, and which replace, reduce, and refine the use of animals. These methods are used to understand biologic mechanisms underlying toxicological processes, for pre-market evaluation of new products, and to generate hazard identification and dose-response relationship information for health and environmental hazard classification and risk assessment purposes. Depending upon the outcome of the assessment, industry and regulatory agencies may implement appropriate prevention and risk management practices to protect public health and the environment. Requirements for the use of new testing methods are twofold: (1) validation, i.e., determination of the scientific validity of a method for a proposed use, and more importantly, (2) acceptance, i.e., determination that a proposed method is acceptable to a regulatory agency. This report describes recommended criteria and processes for the validation and regulatory acceptance of new and revised toxicological testing methods, and presents a set of recommendations to enhance the development and implementation of testing methodologies both nationally and internationally.

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) determined that this report should be applicable to all proposed toxicological testing methods, including those termed "alternatives." This decision was based on the premise that the validation and regulatory acceptance of test methods considered "alternatives" should be no different than for other test methods. For purposes of this Report, alternative test methods have been defined as those that incorporate some aspect of reduction, refinement, and replacement of animal use. These methods:

- result in the reduction of the total number of animals required;

- incorporate refinements of procedures to lessen or eliminate pain or distress to animals and enhance animal well-being; or

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- provide for the partial or total replacement of animals with non-animal systems, or the replacement of an animal species with a phylogenetically lower species (e.g., a mammalian species replaced by an invertebrate species).

Standard validation and regulatory acceptance criteria to guide scientists in the development of new toxicological testing methods have not been readily available from Federal agencies. This Report provides guidance on the principles that should be followed in the validation of a new method and clarifies the critical elements that should be included in the submission of a proposed new method for agency approval. Such guidance should facilitate the evaluation of new methods by regulatory agencies and enhance the likelihood of acceptance of scientifically valid methods.

Validation Criteria

For a new test method to be scientifically valid as a substitute, adjunct, or screening method, it must meet some minimum criteria. These criteria will vary with the method and its proposed use, and will include:

1. The relationship of the test method to the biologic effect of interest must be described. While the relationship may be mechanistic or correlative, mechanistically-based tests are generally preferred. The validation of correlative tests may be somewhat different and more difficult to accomplish than the validation of mechanistic methods.

2. A formal detailed protocol must be provided, describing the test method, the material needed for the conduct of the test, and data evaluation criteria. This should include a clear statement of purpose, a description of the problem and endpoint addressed by the procedure, a description of what is measured, and a listing of the species to which the test results are applicable. The description of the procedure should provide assurance of strict adherence in the validation study, and data analysis and decision criteria.

3. Repeatability and reproducibility of the test method should be demonstrated within and among laboratories. Data should be provided describing the level of inter- and intra-laboratory variability.

4. The test method's performance should be demonstrated on a series of coded reference chemicals or test agents representative of the types of substances to which the procedure will be applied.

5. For a proposed substitute test method, sufficient data should be provided to compare the performance of the proposed test to that of the test it is designed to replace. The performance of test methods should be evaluated in relation to existing toxicity data and experience in the relevant target species.

6. *In vitro* or other non-animal test methods may not replicate all of the metabolic processes relevant to chemical toxicity that occur in an *in vivo* test method. The limitations of an *in vitro* method to address *in vivo* metabolism and disposition should be discussed.

7. The complete data, including data from the testing of coded chemicals and controls, must be available for review. Laboratory records should be maintained in accordance with Good Laboratory Practices procedures.

8. Detailed test protocols, with results, should be published in an independent, peer-reviewed vehicle, or have other evidence of independent scientific review of the procedure.

Because tests can be designed and used for different purposes by different organizations, and with different categories of substances, the determination of whether a specific test method is considered by an agency to be "validated" must be made on a case-by-case basis.

Regulatory Acceptance Criteria and Considerations

In order for a method to be considered for acceptance by a regulatory agency, the following criteria should generally be met:

1. The method is accepted by the scientific community.

2. The method adequately predicts the endpoint of interest in that it demonstrates either a linkage between (a) the new test and an existing test method, or (b) the new test and effects in the target species.

3. There is a defined protocol with standard operating procedures, a list of operating characteristics and criteria for judging test results.

4. There are adequate testing data for chemicals and products representative of those administered by the regulatory program or agency.

5. The method generates data as good or preferably better for hazard identification and/or dose-response assessment than the existing test for which it is proposed to be a substitute.

6. The specific strengths and limitations of the test are clearly identified and described.

7. The test method can be readily transferred among properly equipped and staffed laboratories; that is:

   - it is relatively insensitive to minor changes in protocol;
   - the level of training and expertise required to conduct the test is reasonable;
   - the equipment and supplies needed are relatively easy to obtain;
   - the method is cost-effective;
   - the time to conduct the test is not unreasonable in relation to the test results obtained.

8. The test is likely to be used by industry.

9. The test can be harmonized with other agencies and international groups.

10. The method has been or is likely to be accepted internationally.

Regulatory Acceptance Process

The Committee found that the processes for the evaluation of new methods for regulatory acceptance are not consistent among programs and agencies. There is also no established process for coordinating the review of methods proposed to or by one Federal agency among other agencies that may find the method useful. The recommendations relating to regulatory acceptance that follow are directed at the development of a consistent process for evaluating new methods for regulatory acceptance. Appropriate expertise is essential to adequately and objectively evaluate a new method due to the rapid development of

It should be noted that the acceptance process involves receipt and consideration of input from interested parties. This includes evaluation by stakeholders (e.g., test sponsors and users, groups affected by regulatory decisions) through such things as workshops and public notices in the *Federal Register*, and independent peer review by knowledgeable persons unencumbered by the outcome of the review. Both are integral parts in determining the acceptability of a method (Balls et al., 1990b).

PROCESS OF REGULATORY ACCEPTANCE

Agencies with regulatory programs should promote opportunities for interagency and international harmonization to broaden the scientific and policy base, share limited resources, reduce review time and effort for any single authority, decrease testing demands on industry, and reduce reliance on animal testing. Acceptance of methods by international organizations (e.g.; OECD, UN Transport) will also aid in achieving acceptance by the U.S. government.

Depending on its application, there are several routes that the a method may take within the Federal Government. Some methods will need acceptance by several agencies, while others will be applicable to a single agency, and still others will only be applicable to one program within an agency.

For methods that are designed to be used in testing paradigms within several agencies an interagency committee should be established to facilitate and formulate a path for its acceptance into the regulatory arena. The committee might be composed of representatives from each of the agencies involved. That group could either operate alone or incorporate outside consultants. Other options would be to utilize a consensus conference or public workshop to reach agreement on the applicability of the method. The potential of combining members from science advisory groups from relevant agencies might also be explored.

For methods that will be submitted to only one agency or one program within an agency, a specific process for regulatory acceptance needs to be developed. Suggested options are to use an agency's external science advisory group to review the method or present it to an in-house committee of scientists or both.

RECOMMENDATIONS FOR REGULATORY ACCEPTANCE PROCESSES

Test method acceptance among regulatory agencies has largely been an *ad hoc* procedure. It is recognized that there is a need to improve the process to make it more

streamlined and efficient. Remarks are directed to three general needs—openness to new methods, communication, and harmonization.

Openness to New Test Methods

Issues

The scientific bases and understanding of chemically induced adverse health effects are developing at a rapid rate. Regulatory agencies have missions to protect human health and the environment, and they need to keep an open mind concerning new and revised test methodologies that may apply to their programs. Some methods that show promise are alternative test methods that reduce, refine, or replace animal use. Both mechanistic and correlative methods are being developed.

Recommendations

1. Test methods should be periodically reviewed and, where appropriate, revised in light of scientific and policy developments.

2. Current efforts should be continued and expanded to incorporate scientifically valid and useful alternative test methods into regulatory testing strategies.

3. While both correlative and mechanistic tests can be validated and found acceptable, future emphasis should be placed upon those methods with a biological basis relevant to the biological/health effects of concern.

Improved Communication

Issues

All too often there is inadequate communication among programs of an agency, among Federal agencies, and among international bodies that deal with testing guidance. Federal agencies have not effectively communicated their testing needs to others outside of government. Scientists involved with test method development, validation, and the assessment of the validation status of methods do not always solicit regulatory input during their activities and vice versa.

Recommendations

1. Federal regulatory agencies should establish consistent processes and criteria, as appropriate, for the acceptance of new and revised toxicological test methods and communicate them to interested parties.

2. Federal regulatory programs should solicit input from other programs and agencies as they develop and modify test guidelines of general interest.

3. A Federal interagency committee on test methods should be established to serve as a forum for exchange of information and coordination of test methods and related activities. This committee would have as its objectives both consistency within government agencies and international acceptance of alternative methods.

4. Assessment of the validation status of a particular test method should include Federal agencies.

5. Validation and regulatory acceptance should include the opportunity for input by interested stakeholders inside and outside of government (e.g., test sponsors, regulated industry, regulatory and research agencies).

6. Validation and regulatory acceptance should include independent scientific peer review by persons expert in the field, knowledgeable in the test method, and unencumbered with the outcome of the evaluation.

7. Where appropriate, the regulatory acceptance of new and revised test methods by agencies should be communicated to scientists and to various national and international organizations, for example in journals, workshops, the *Federal Register*, and by other means.

8. Agency staff should understand and apply the criteria and processes for acceptance of a test method during the review process.

9. Agency staff should be adequately trained in the evaluation of data from newly accepted test methodologies.

Harmonization

Issues

Regulatory programs often unilaterally approve test methodologies that may also apply to other programs and agencies. Although there are organizations dealing with the preparation of international guidelines for toxicological testing, they often apply to only some of the chemicals in commerce (e.g., industrial chemicals, pharmaceuticals, etc.), and there is incomplete coordination among these international bodies. An impediment to

domestic interagency harmonization is a lack of coordination across international organizations.

Recommendations

1. Proposed new or revised test methods relevant to the needs of more than one program or agency should be harmonized, as appropriate.

2. Harmonization of hazard classification may be necessary before test guidelines can be harmonized.

3. U.S. agencies should attempt to harmonize guidelines through international organizations, e.g., the OECD, as appropriate.

4. U.S. agencies should encourage harmonization of test guidelines across international organizations, e.g., between U.N. Transport and OECD, as appropriate.

Figure 2.

New Toxicological Methods Process Flow Diagram